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Abstract

Background and Aims: The possible contribution of iron to cardiovascular complications of type 2 diabetes (T2D) has been scarcely investigated. We aimed to study whether serum ferritin is linked to prevalent/incident cardiovascular disease (CVD) in T2D.

Methods: The prevalence of coronary heart disease (CHD), cerebrovascular disease (CEVD) and CVD were evaluated in the SIDIAP study (n=38,617) and prevalence and 7 year incidence were analysed in the Edinburgh Type 2 Diabetes Study (ET2DS)(n=821). Logistic and Cox regressions were used to describe associations between serum ferritin and the CVD adjusting for confounding variables.

Results: Increase of 1 SD unit in log-ferritin was associated with lower CVD prevalence in fully-adjusted models [ET2DS Odds ratio (OR) 95% confidence interval (CI): 0.81(0.68-0.96), *P*=0.018; SIDIAP study: 0.91(0.88-0.94), *P*<0.001). In ET2DS, ferritin in the highest (vs. the lowest) quintile was associated with lower incidence of CVD [fully adjusted HR 95% CI: 0.46(0.26-0.83), *P*=0.010]. This association persisted after removing subjects with CVD at baseline (n=536) [HR 95% CI: 0.34 (0.14-0.81), *P*=0.016].

Conclusions: Low iron status was associated with CVD risk in T2D. This pattern was consistent in populations at different cardiovascular risk. Low iron status seems to be harmful for cardiovascular health in T2D and it may be a target for intervention.

Keywords: ferritin, cardiovascular disease, type 2 diabetes

Introduction

A positive association between increased body iron stores, estimated as serum ferritin, and incidence of type 2 diabetes (T2D) has been consistently reported (1, 2)]. In the general population, iron has been hypothesised to promote the development of the atherosclerotic plaque through increased oxidative stress as a consequence of the pro-oxidant properties of iron (3)]. Although iron excess has been the most reported iron imbalance associated with insulin resistance and development of diabetes and metabolic syndrome in general populations, its association with cardiovascular disease is still controversial. Iron excess reflected by high ferritin levels was positively associated with incident coronary heart disease in Finnish men but subsequent studies have failed to replicate this association (4)].

On the other hand, a significant negative association between transferrin saturation and development coronary artery disease or myocardial infarction has been recently identified in the general population in a recent meta-analysis. Puzzlingly, no significant association was found with serum ferritin (5)]. Importantly, a recent Mendelian randomisation study reported that genetic markers related to systemic high iron status (rs1800562 and rs1799945 in the HFE gene and rs855791 in TMPRSS6 gene) reduced the risk of coronary heart disease (CHD) in the general population(6)].

Little attention has been paid to the possible involvement of iron in chronic diabetic complications. Iron imbalance could potentiate the risk for cardiovascular outcomes in people who already have T2D. So far, only one cross-sectional study of 424 people has tested this hypothesis. The authors reported an apparently paradoxical decreased prevalence of macroangiopathy in men with T2D and increased serum ferritin levels but adjustment for covariates was not performed (7)].

In the light of the above, we aimed to characterise the association between serum ferritin with (CHD) and cerebrovascular disease (CEVD) with adjustment for covariates in two different cohorts of people with type 2 diabetes, one from Scotland-UK and other from Catalonia-Spain using both cross-sectional and prospective study designs. This multi-cohort approach was used to evaluate whether any association was consistent in different populations even despite different cardiovascular risk, which is higher in British (8, 9)] than in Mediterranean populations (10)].

Patients and Methods

Study population

ET2DS

Methods of the Edinburgh Type 2 Diabetes Study (ET2DS) have been described in full previously (11)]. The ET2DS is an ongoing prospective study with available data for eight years follow-up. Briefly patients with type 2 diabetes aged 60–75 years were selected at random from the Lothian Diabetes Register, a comprehensive register of patients with diabetes living in Lothian, Scotland, UK, which was established in 2001. Baseline attendees (n=1,066) have previously been shown to be representative of all those randomly selected to participate (n=5,454), and therefore representative of the target population of older people with type 2 diabetes living in the general population. There was a liver assessment clinic attended by 939 participants at year 1, and ferritin levels were measured in 876 of those participants. Therefore year 1 was used as baseline for this study and follow-up for the present analysis was of seven years. Of the 876 subjects with ferritin measurements we excluded 55 individuals with missing values for covariates leaving a remaining final sample of 821 participants.

SIDIAP study – Primary Care Centers in Catalonia

This cross-sectional study is based on the SIDIAP system which a computerized database containing anonymized patient records for the 5.8 million people registered with a GP in the Catalan Health Institute. The SIDIAP database includes data from a software called ECAP used by general practitioners to record clinical information on patients (demographics, consultations with GPs, diagnoses, clinical variables, prescriptions, and referrals), laboratory test results, and medications obtained from the pharmacists (provided by the CatSalut database) (12)]. Methods of SIDIAP study have been described in full previously (12, 13)]. Of 318020 individuals with T2D aged 30-99 years registered in the SIDIAP database in 2011, 62002 had at least one measurement of ferritin during this year. The final sample for the analysis was composed of 38617 individuals after excluding cases with missing values for covariates.

CVD events

The ET2DS has data on prevalent and incident CVD events, and the SIDIAP study on prevalent CVD only. The ET2DS has had eight years follow-up. Prevalent and incident events up to year 4 were collected using a combination of record linkage (hospital discharge and death certification data), repeat self-report and GP questionnaires, repeat ECG and inspection of clinical notes. Incident events between year 4 and year 8 were collected using a combination of record linkage and inspection of clinical notes. Data linkage was undertaken, via the National Health Service National Services Scotland, to Scottish Morbidity Record (SMR01) general and acute inpatient discharge records using ICD-10 (www.who.int/classifications/icd/en/) (and related ICD-9 [www.icd9data.com/2007/Volume1]) codes. CVD cases of the SIDIAP study were defined on the basis of record linkage.

Ischemic heart disease or coronary heart disease (CHD) was in terms of angina or myocardial infarction (ICD-10 codes I20, I21, I22, I23). The SIDIAP study also included the

category of “other acute ischaemic heart disease” (ICD-124). Cerebrovascular disease (CEVD) was in terms of stroke (ICD-10 codes I63, I64) and transient ischaemic attack (TIA) (ICD-10 code G45). The ET2DS also included ICD-10 code 161 for intracerebral hemorrhage, and the SIDIAP study also considered the ICD-10 code G46 on vascular syndromes of brain in cerebrovascular diseases. In the ET2DS fatal cases of myocardial infarction and stroke were according to have the respective ICD-10 codes as primary cause of death. The ET2DS also used inspection of clinical notes to confirm CVD cases (prevalent or incident) if non-primary ICD-10 code for CHD or CEVD was reported, except in the cases of angina.

Clinical and biochemical variables

The SIDIAP included similar clinical and biochemical variables than the ET2DS, with exception of fibrinogen, CRP, and transaminases levels, and estimation of liver disease which were available in the ET2DS only. In the SIDIAP study white blood cells count was used as a measure of inflammation, and triglyceride levels was also available. Measurements of clinical variables of weight, height , blood pressure, estimation of liver disease and biomarkers [haemoglobin levels, HbA1c, fasting glucose, HDL-cholesterol, total cholesterol, triglycerides, white blood cells count, C reactive protein (CRP), fibrinogen, transferases levels and estimated glomerular filtration rate (eGFR] were performed as described elsewhere for both studies (13-15)]. In both studies ferritin levels were measured by immunoturbidimetry (intra- and interassay coefficients of variation <8).

Data analyses for the ET2DS and SIDIAP study

Study variables were described as median (interquartile ranges) or proportions if categorical. Ferritin levels were described using Z scores of logarithm of ferritin values (continuous variable) and also as sex-specific quintiles (categorical variable), in order to evaluate linear and non-linear relationships. Z score of log-ferritin was chosen instead of ferritin or log-

ferritin alone to provide information about changes in risk of the outcomes by increasing standard deviations of the iron marker. In the case of quintiles of ferritin, since iron overload has been the most commonly reported for cardio-metabolic risk in general population, the first quintile was used as reference. Baseline cross-sectional associations between ferritin and prevalent cardiovascular diseases were tested by using logistic regression. Longitudinal associations were evaluated by using Cox regression, and proportional hazards assumption was tested by Schoenfedl residuals test and graphical method. For this analysis the years of follow-up were calculated from date of attendance at year 1 (baseline of this study) to the first of date of CVD event, death or end of December 2014. For individuals who developed both CHD and CEVD, the years of follow-up for incident or recurrent CVD were derived using the date of the first event. We conducted logistic and Cox regressions creating adjustment models with covariates associated with the outcomes in univariate analyses at a level of statistical significance <0.1 and which remained statistically significant with P< 0.05 in the multivariate regression using a backward elimination approach. The covariates tested were chosen on the basis of possible influence in cardiovascular risk and/or serum ferritin levels, and were: age (at baseline ET2DS), sex, duration of diabetes, use of specific anti-hyperglycaemic agents, treatment with insulin, lipid-lowering drugs, blood pressure-lowering drugs, smoking(ever/never), alcohol consumption (ever/never), body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), HbA1c, fasting glucose, HDL-cholesterol, total cholesterol, C reactive protein (CRP), fibrinogen, liver enzymes, liver disease, estimated glomerular filtration rate (eGFR), haemoglobin levels, and antiplatelet drugs. Anaemia as haemoglobin levels < 13 g/dL in men/ <12 g/dL in women(16), was alternatively tested instead of haemoglobin levels. The cross-sectional analysis in the SIDIAP study included the above covariates except fibrinogen, CRP, fasting glucose and transaminases levels, but included white blood cells count (WBC) and triglycerides levels. Additionally we performed the regressions adjusting for all the covariates of potential influence in models of increasing complexity, regardless of statistical significant association

with the outcomes, and these findings are presented in the supplementary file. More details about the methodology for statistical analyses are described in the supplementary file. A p value <0.05 was set as statistically significant. All the analyses were performed using STATA software 14.0.

Results

Cross-sectional associations

As expected, serum ferritin levels were significantly lower in women than in men in both the ET2DS [median, interquartile range 56 (23-100) µg/L vs. 96.5 (45.5-179.2) µg/L and SIDIAP study [46.1 (21-101) µg/L vs. 97.0 (39.0-208) µg/L (*P*<0.0001). In both studies, CVD was more prevalent in men than in women [In *ET2DS*: CVD 41% vs. 28.1% (P<0.001), CHD 36.3% vs. 24.3% (P<0.001), and CEVD 10.9 vs. 6.3% (P=0.018); In *SIDIAP* study: CVD 28.5 % v. 16.3% (P<0.001), CHD 22.0 % v. 11.4% (P<0.001), and CEVD 9.1 v. 5.9 % (P<0.001)]. Serum ferritin levels were decreased with longer duration of diabetes in the ET2DS and the SIDIAP study, Figure 1 in the Supplementary file.

SIDIAP study

Study variables of the 38,617 patients with T2D are described by sex-specific quintiles of ferritin in (Table 1). The proportion of subjects reporting alcohol intake increased across the quintiles of serum ferritin. In this large population, the proportion of individuals taking oral anti-hypoglycaemic and antiplatelet drugs decreased across quintiles of ferritin. The same occurred with values of HbA_{1C}, SBP and eGFR, and with the number of cases with insulin, antihypertensive, and lipid-lowering treatments. There were dose-response relationships between serum ferritin concentration, haemoglobin levels, serum total cholesterol and

triglycerides level. Cases of CHD, CEVD and CVD, were increased in the lower quintiles of ferritin (Table 1).

In subjects from the SIDIAP study, ferritin as continuous variable or as quintiles was significantly inversely related to the opportunity of having CVD (Table 2).

ET2DS cohort

Study variables at baseline are described by sex-specific quintiles of ferritin in Table 3. Values of haemoglobin levels, blood glucose, CRP, transaminases, and DBP increased across quintiles of ferritin. As in the SIDIAP study, there were higher proportions of individuals reporting alcohol consumption across increasing levels of ferritin, and use of oral anti-hyperglycemiant drugs and aspirin decreased across increasing levels of ferritin. CVD prevalence tended to decrease across ferritin quintiles (P for trend=0.092) (Table 3).

Table 4 shows cross-sectional associations of ferritin with prevalent cardiovascular disease. In terms of ferritin as quintiles, comparing the lowest v. highest category, there was an inverse association of ferritin levels with CVD marginally significant [Odds ratio (OR) 95% confidence interval (95%CI) : 0.62(0.37-1.04) ; P=0.073] after adjustments. When ferritin levels as a continuous variable were introduced in the same adjustment model, the inverse association with CVD became more evident [OR 95%CI : 0.81(0.68-0.96); P= 0.018].

Comparisons of the study variables between the SIDIAP study and the ET2DS are described and shown in the online Supplementary file and Table 1 in the Supplementary file, respectively.

Longitudinal findings (ET2DS)

After a mean follow-up of 6.1 ± 1.7 years [median 6.7, interquartile range 6.3-6.9] the number (proportion) of people with incident or recurrent outcomes were 123 (15%) for either CHD or CEVD, 78 (9.5%) for CHD, and 54 (6.6%) for CEVD. Nine people developed both types of events during the follow-up. When considering participants without disease at baseline (n=536) with a mean follow-up of 6.2 ± 1.5 years [median 6.7 (6.4-7.0)], there were 60 patients (11.2%) with incident CVD, 38(7.1%) with incident CHD, and 25(4.7%) with incident CEVD. There was no significant difference by sex in proportion of incident or recurrent cases for CVD (57 men, 66 women), CHD (34 men, 38 women) or CEVD (32 men, 22 women) during follow-up. There were 132/821 (16.1%) deaths during the follow-up, with 26/132 (19.6%) cases due to CVD, of which 19 were attributable to CHD.

Hazard ratios (HR) and 95% confidence intervals (CI) for cardiovascular disease are shown in Table 5 according to quintiles of ferritin. In a full-adjusted model that included CVD at baseline, when comparing the extreme quintiles, ferritin was inversely associated with development of CVD (HR 95% CI 0.46[0.26-0.83] P=0.010]. When restricting the analyses to 536 people without prevalent cardiovascular disease at baseline, the longitudinal inverse associations between ferritin levels and CVD persisted [HR 95% CI 0.34(0.14-0.81); P=0.016] (Table 5).

The findings of inverse association between serum ferritin and CVD previously described were also obtained in adjustment models of increasing complexity with all the covariates [ET2DS HR(95%CI)= 0.50(0.27-0.94) P=0.031; SIDIAP OR(95%CI)= 0.61 (0.55-0.66) P<0.001] (Tables 2-7 in the supplementary file). Specific associations of serum ferritin with CHD and CEVD are also shown in the above tables of the supplementary file. These associations were consistent with the associations with CVD, except ferritin-CEVD in the ET2DS, presumably due to lower statistical power.

Iron deficiency was associated with CHD and CVD in the SIDIAP study and with incident CHD in women of the ET2DS (more details in Supplementary file). Comparisons between the included and excluded subjects of the SIDIAP study (Table 8 in the Supplementary file), and additional adjustments and sensitivity analyses mentioned in the data analysis section (Supplementary file), are described in the Supplementary file.

Discussion

We have described independent inverse cross-sectional and prospective association between ferritin levels and cardiovascular disease in representative samples of older people with type 2 diabetes from Catalonia-Spain and from Scotland. The multivariate adjusted estimates are similar to the previous unadjusted cross-sectional observations by Hermans et al. (7)], and suggests that the inverse association between ferritin levels and incident or recurrent CVD in people with type 2 diabetes is also observed in larger samples, in a prospective design, and in populations at different cardiovascular risk.

Low iron status or iron deficiency could explain higher cardiovascular risk in the subjects affected by diabetes of our study. Besides the cross-sectional evaluation by Hermans et al. (7)], there are few other studies to which we can compare our findings in T2D. Iron depletion by phlebotomy in people with diabetes and peripheral arterial disease (n=636, controls 641) did not decrease all-cause mortality or incidence of cardiovascular events (17)]. In other study, both low and high ferritin levels, and high soluble transferrin receptors levels were associated with 5-year all-cause mortality rate in 287 people with diabetes and stable coronary artery disease (18)]. Current findings are supported by recent studies in the general population describing inverse associations between iron markers and development of CHD

in meta-analyses of existing studies (5)] and in a mendelian randomization approach (6)]. However, the underlying mechanisms are still unclear. Meanwhile, the ferritin-CEVD association may require a relatively high statistical power, since this was only observed in the large population of the SIDIAP study.

The inverse association between iron status and CVD could also suggest that high iron status may be a protective factor against CVD in people with diabetes. Hermans et al. claimed this hypothesis by highlighting lesser known pleiotropic anti-oxidant properties of the ferritin protein itself potentially acting as a compensatory response to inflammation in type 2 diabetes (7)]. Findings in murine models of oxidative stress-induced synthesis of iron proteins in diverse cell lines support this idea since inflammation is linked to increased oxidative stress (19)]. However, in our study the association was unaffected when adjusting for inflammatory markers such as CRP or fibrinogen levels.

Our findings may also be associated to pleiotropic effects of ferritin on blood vessel formation. An in vitro study showed that ferritin stimulates proliferation of endothelial cells via inactivation the HKa, which is a potent angiogenesis inhibitor (20)]. Therefore a higher iron status in T2D, in terms of high ferritin, might preserve integrity of vascular tissue decreasing the risk for cardiovascular injuries.

Our finding that high iron status appears to be a protective factor for cardiometabolic risk in T2D could be also related to the course of T2D rather than direct causal beneficial effects of ferritin. Systemic metabolic alterations triggered by T2D can encompass several regulatory pathways of iron metabolism affecting ferritin levels, circulating iron and ultimately the cardiovascular risk attributable to iron. A recent study in mouse and *in-vitro* models confirmed that starvation promotes raising of serum ferritin levels, hepatic and splenic iron retention and decreased serum iron via gluconeogenesis, a persistently active pathway in

T2D, with subsequent increasing levels of the hormone hepcidin (21)]. In other words, high ferritin levels in T2D could reflect circulatory iron depletion which would avoid pro-oxidant iron effects in vascular function. It is also likely that type 2 diabetes influences iron proteins through other mechanisms and/or that there is residual confounding from factors not measured in the present study.

To the best of our knowledge this is the first longitudinal study and large cross-sectional study evaluating body iron stores and cardiovascular complications in T2D. Additional strengths were the use of large and representative samples of people with T2D and a broad set of covariates to verify an independent association.

CRP, haemoglobin and fibrinogen levels were measured at the original baseline of the ET2DS, one year before ferritin was measured. However, CRP and haemoglobin levels were related in a dose-response manner to ferritin quintiles, which is in line with the positive associations between these markers that have been observed in diverse populations(22, 23)], although with variable and modest strength in the case of CRP (24-26)].

There were slight differences in definitions of CHD and CEVD in the ET2DS and the SIDIAP study due to predetermined ways of establishing variables of disease in each study. This may have biased findings on the ferritin-CEVD association which was significant in the SIDIAP study only, but as mentioned previously, this discrepancy would be more related to statistical power. On the other hand, the ferritin-CHD association was consistent in both studies. There is a possible selection bias in the SIDIAP cohort because serum ferritin measurement was more probably performed in subjects suspected of having iron disorders, and excluded individuals had different characteristics from those included. However, these issues may not have had substantial influence since adjustment for anaemia did not affect the significance of the associations, and the association between ferritin and CVD was

present in included and excluded individuals. There was also difference in the age ranges of the studies evaluated, but our sensitivity analysis using identical ranges did not alter our conclusions. It will be interesting to study whether the observations obtained in this study stand only for T2D patients or extend to other conditions in which a chronic oxidative stress state is prominent.

Conclusion

Low iron status seems to be harmful for cardiovascular health in T2D. Although the underlying mechanisms for this association remain unclear and further studies are required, subclinical/clinical inflammation, body mass index, concomitant treatment, cardiovascular risk factors, anaemia, aspirin intake and duration of diabetes, do not appear to be primary explanatory variables. It is also plausible that residual confounding could contribute to the association.

Conflict of interests: No conflicts of interest to disclose

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Authors' contributions: M.F.S.O. conceived and designed the study, analysed data and wrote the first draft of the manuscript. S.M. researched data, supervised the analysis, and edited the manuscript. A.H.P. researched data and edited the manuscript. M.F.B, J.F.N, M.MC, J.B.F, X.M.T, D. M, and W.R., edited the manuscript and contributed to the

discussion. S.H.W. supervised the analysis, edited the manuscript and contributed to the discussion. M.W.J. and J.F.P. are the principal investigators of the ET2DS and edited the manuscript. J.M.F.R. conceived and designed the study, edited the manuscript and contributed to the discussion.

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Table 1. Characteristics of people with type 2 diabetes by sex-specific quintiles of ferritin level in the SIDIAP study(n=38617)

	Ferritin (µg/L)					
	Q1 (n=7712)	Q2(n=7740)	Q3(n=7724)	Q4(n=7732)	Q5(n=7709)	P for trend
Sex Male/Female n	3283/4429	3275/4465	3296/4428	3290/4442	3279/4430	
Ferritin levels range by sex (µg/L)						
Men (n=14757)	*3.0-31.3*	*31.4-69.9*	*70.0-131.1*	*131.2-244*	*244.1-3507.8*	
Women (n=19847)	*1.0-17.7*	*17.8-34.0*	*34.1-62.0*	*62.1-121*	*121.1-3682.1*	
Variables						
Age (years)	74 (65-79)	75 (66-80)	74 (65-80)	72 (63-80)	71 (63-79)	<0.001
Duration of diabetes (years)	7.8 (5.4-11.5)	7.7 (5.2-11.1)	7.5 (4.6-10.8)	7 (3.6-9.9)	6.2 (2.6-8.8)	<0.001
Ferritin (µg/L)	13.3 (9.9-17)	31.6 (23.9-44.6)	57.6 (44.6-92)	110.1 (82-168.2)	273 (178.3-390.9)	<0.001
Haemoglobin (g/dL)	12.0(11.0-13.0)	12.6(11.7-13.7)	12.9(11.9-14.0)	13.2(12.1-14.3)	13.3(12.0-14.5)	<0.001
HbA1C (%)	7.1 (6.5-8)	7 (6.4-7.9)	6.9 (6.3-7.8)	6.8 (6.3-7.7)	6.8 (6.2-7.6)	<0.001
White blood cells count (cells/µL)	7.2 (6-8.5)	7.2 (6-8.5)	7.2 (6-8.6)	7.1 (6-8.5)	7.2 (6-8.6)	0.364
Smoking [ever]n(%)	2463 (31.9)	2202 (28.4)	2220 (28.7)	2274 (29.4)	2328 (30.2)	<0.001
Alcohol consumption [ever] n(%)	1338 (17.3)	1374 (17.8)	1567 (20.3)	1706 (22.1)	1897 (24.6)	<0.001
BMI (Kg/mts^2)	29.4 (26.5-33)	29.4 (26.3-32.8)	29.3 (26.4-32.9)	29.3 (26.4-32.9)	29.3 (26.3-32.9)	0.217
SBP mmHg	135 (125-142)	134 (125-141)	134 (125-141)	135 (125-141)	134 (125-140)	<0.001
DBP mmHg	73 (67-80)	73 (66-80)	74 (67-80)	73 (68-80)	75 (68-80)	<0.001
Total cholesterol mg/dl	4.52 (3.93-5.14)	4.57 (3.98-5.24)	4.62 (4.0-5.24)	4.68 (4.06-5.35)	4.73 (4.0-5.43)	<0.001
HDL-cholesterol mg/dl	1.21 (1.03-1.44)	1.21 (1.03-1.44)	1.24 (1.03-1.47)	1.24 (1.03-1.47)	1.21 (1.03-1.44)	0.403
Triglycerides mg/dl	1.43 (1.06-1.97)	1.43 (1.06-1.97)	1.41(1.03-1.96)	1.42 (1.05-1.97)	1.51 (1.10-2.10)	<0.001
eGFR, mL/min/1.73 m^2	60 (58.5-60)	60 (52.9-60)	60 (52.3-60)	60 (53.5-60)	60 (53.4-60)	<0.001*
Antiplatelet drugs n(%)	3980(51.6)	3814(49.3)	3500(45.3)	3104(40.1)	2527(32.8)	<0.001
Oral hypoglycaemic drugs n(%)	6503 (84.3)	6185 (79.9)	5952 (77.1)	5575 (72.1)	5116 (66.4)	<0.001
Antihypertensive drugs n(%)	6312 (81.8)	6506 (84.1)	6284 (81.4)	6066 (78.5)	5896 (76.5)	<0.001
Insulin therapy n(%)	2026 (26.3)	1997 (25.8)	1808 (23.4)	1606 (20.8)	1378 (17.9)	<0.001
Lipid-lowering drugs n(%)	4961 (64.3)	4869 (62.9)	4944 (64)	4669 (60.4)	4327 (56.1)	<0.001
CHD n(%)	1504 (19.5)	1429 (18.5)	1296 (16.8)	1034 (134)	880 (11.4)	<0.001
CEVD n(%)	668 (8.7)	626 (8.1)	572 (7.4)	511 (6.6)	435 (5.6)	<0.001
CVD n(%)	2008 (26)	1894 (24.5)	1723 (22.3)	1441 (18.6)	1220 (15.8)	<0.001

Data are median (interquartile range) or prevalence (95% CI). P for trend across quintiles by Jonckheere-Terpstra Test (continuous variables) and χ^2 tests (categorical variables). Q, quintile. CI, confidence interval. HDL-C, HDL cholesterol. BMI, body mass index. eGFR, estimate glomerular filtration rate. HbA1C, glycosylated haemoglobin. SBP, systolic blood pressure. DBP, diastolic blood pressure.*Positive trend is more

evident in terms of rank values.

Table 2. Multivariate model* explaining prevalent cardiovascular disease in the SIDIAP study

	OR 95%CI	P value
Ferritin (Z score of log-ferritin)	0.91(0.88-0.94)	P<0.001
Ferritin (µg/L)		
Quintile 1	1.0(Reference)	
Quintile 2	**0.91(0.84-0.99)**	**0.036**
Quintile 3	**0.87(0.80-0.95)**	**0.002**
Quintile 4	**0.77(0.70-0.84)**	**<0.001**
Quintile 5	**0.72(0.65-0.79)**	**<0.001**
Age (years)	**1.02(1.02-1.03)**	**<0.001**
Sex Male/Female n	**1.65(1.54-1.77)**	**<0.001**
Smoking [ever]	**1.07(1.03-1.11)**	**<0.001**
Alcohol consumption [ever]	**0.94(0.89-0.99)**	**0.020**
BMI (Kg/mts^2)	**0.98(0.98-0.99)**	**<0.001**
SBP mmHg	**0.99(0.99-0.99)**	**0.032**
DBP mmHg	**0.98(0.98-0.99)**	**<0.001**
HDL-cholesterol mg/dL	**0.98(0.97-0.98)**	**<0.001**
LDL-cholesterol mg/dL	**0.99(0.99-0.99)**	**<0.001**
Triglycerides mg/dl	**0.99(0.99-0.99)**	**0.014**
eGFR, mL/min/1.73 m^2	**0.99(0.99-0.99)**	**0.014**
Oral hypoglycaemic drugs	**0.65(1.04-1.19)**	**<0.001**
Antihypertensive treatment	**2.04(1.84-2.26)**	**<0.001**
Insulin therapy	**1.11(1.04-1.19)**	**0.001**
Lipid-lowering treatment	**2.09(I1.95-2.23)**	**<0.001**
Antiplatelet treatment	**5.28(4.96-5.63)**	**<0.001**

* Model on the basis of variables selected from a previous univariate analysis (P<0.1) and which remained significant in the multivariate model after a backward elimination approach (P<0.05).
The variables tested in the univariate analysis were the following: age, sex, duration of diabetes, use of specific anti-hyperglycaemic agents, treatment with insulin, lipid-lowering drugs, blood pressure-lowering drugs, smoking(ever/never), alcohol consumption (ever/never), body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), HbA1c, triglycerides, HDL-cholesterol, LDL-cholesterol,

total cholesterol, white blood cells count, estimated glomerular filtration rate (eGFR), haemoglobin levels, and antiplatelet drugs. Anaemia was alternatively tested instead of haemoglobin levels.
CI, confidence interval. BMI, body mass index. SBP, systolic blood pressure. DBP, diastolic blood pressure. CRP, C reactive protein. GGT, gamma-glutamyl transpeptidase. ALT, Alanine aminotransferase. eGFR, estimate glomerular filtration rate.

Table 3. Baseline characteristics of people with type 2 diabetes by sex -specific quintiles of ferritin level in the ET2DS (n=821)

	Q1 (n=162)	Q2(n=164)	Q3(n=167)	Q4(n=164)	Q5(n=164)	P for trend
Sex Male/Female n	84/78	85/79	85/82	84/80	84/80	
Ferritin levels range by sex (µg/L)						
Men (n=422)	7-36	37-74	75-123	124-198	199-1095	
Women (n=399)	5-20	21-38	39-69	70-119	120-605	
Variables						
Age (years)	69.9(65.5-72.7)	68.4(65.2-71.5)	68.6(65.2-72.5)	68.9(64.8-72.1)	69.7(65.5-72.8)	0.751
Duration of diabetes (years)	9(5-14)	8(4-13)	8(5-11)	7(4-10)	5(3-10)	<0.001
Ferritin (µg/L)	17(11-25.2)	38(28-52)	75(55-98)	124(87-162.5)	246(177.5-338)	<0.001
Haemoglobin (g/dL)	13.4(12.5-14.5)	14.0(12.9-15.1)	14.1(13.2-15.1)	14.5(13.7-15.3)	14.6(13.6-15.5)	<0.001
Glucose mmol/L	6.2(5.0-8.0)	6.5(5.5-7.9)	6.3(5.3-7.5)	6.4(5.4-7.9)	6.7(5.8-8.2)	0.011
HbA1C (%)	7.0(6.4-7.9)	7.0(6.5-7.7)	7.0(6.4-7.5)	7.0(6.4-7.7)	7.0(6.4-7.8)	0.525
Fibrinogen (g/L)	3.5(3.1-4.0)	3.7(3.1-4.1)	3.6(3.0-4.21)	3.5(3.1-3.9)	3.5(3.1-4.0)	0.781
CRP(mg/L)	1.5(0.7-3.0)	1.5(0.7-3.9)	1.8(0.8-4.6)	2.1(1.1-4.3)	2.2(1.0-4.5)	<0.001
GGT(U/L)	13(8-25.5)	14(9.0-23)	17(10-29)	17.5(10-31.7)	24.5(13-44.7)	<0.001
ALT(U/L)	29(24-34)	28(25-36)	31(25-36)	34(27.2-41)	36(28-47)	<0.001
AST(U/L)	27(22-32)	27(24-32)	28(24-33)	29(25-34.7)	33(27-41)	<0.001
Smoking [ever] n(%)	101(62.3)	89(54.3)	101(60.5)	103(62.8)	96(58.5)	0.935
Alcohol consumption [ever] n(%)	124(76.5)	129(78.7)	143(85.6)	136(82.9)	141(86)	<0.001
BMI (Kg/mts^2)	30.8(27.6-34.4)	30.5(26.7-34.1)	30.2(27.3-33.8)	30.6(27.1-35.4)	30.7(27.9-34.0)	0.485
SBP mmHg	132(120-142)	132(122-142)	130(122-140)	133(120-142)	134(124-144)	0.685
DBP mmHg	68(60-74)	68(62-74)	70(62-76)	70(64-76)	70(64-78)	0.031
HDL-cholesterol (mmol/L)	1.3(1.1-1.5)	1.3(1.1-1.5)	1.2(1.1-1.4)	1.2(1.0-1.4)	1.2(1.0-1.5)	0.006
Total cholesterol (mmol/L)	4.1(3.7-4.6)	4.2(3.8-4.7)	4.1(3.6-4.8)	4.3(3.8-4.9)	4.2(3.7-4.8)	0.166
Aspirin consumption n(%)	109(67.5)	111(67.7)	117(70.1)	111(67.7)	103(62.8)	0.208

eGFR, mL/min/1.73 m^2	60(60-65.2)	60(60-71)	60(60-72)	60(60-73)	60(60-68.5)	0.757
Oral hypoglycaemic drugs n(%)	137(84)	134(81.7)	124(74.3)	119(72.6)	93(56.7)	<0.001
Antihypertensive drugs n(%)	145(89.5)	139(84.8)	147(88)	136(82.9)	138(84.1)	0.147
Lipid-lowering drugs n(%)	147(90.7)	132(80.5)	139(83.2)	137(83.5)	136(82.9)	0.168
Insulin therapy n(%)	28(17.3)	25(15.2)	27(16.2)	21(12.8)	22(132.4)	0.250
Liver disease n(%)	2(1.2)	0(0)	1(0.6)	6(3.7)	3(1.8)	0.102
Outcomes at baseline n(%)						
CHD	59(36.4)	48(29.3)	50(29.9)	49(29.9)	44(26.8)	0.104
CEVD	15(9.3)	15(9.1)	12(7.2)	19(11.6)	10(6.1)	0.576
CVD (any cardiovascular event)	67(41.4)	58(35.4)	52(31.1)	55(33.5)	53(32.3)	0.092
Outcomes after follow-up						
Development of CHD	21(13)	12(7.3)	16(9.6)	20(12.2)	9(5.5)	0.107
Development of CEVD	17(10.5)	7(4.3)	10(6.0)	9(5.5)	11(6.7)	0.306
Development of CVD	35(21.6)	17(10.4)	24(14.4)	27(16.5)	20(12.2)	0.168

Data are median (interquartile range) or *n*(%). P for trend across quintiles by Jonckheere-Terpstra Test (continbuous variables) and χ^2 tests (categorical variables). Q, quintile. CI, confidence interval. HDL-C, HDL cholesterol.. BMI, body mass index. GGT, gamma-glutamyl transpeptidase. ALT, Alanine aminotransferase. AST, Aspartate aminotransferase. CRP, C reactive protein. eGFR, estimate glomerular filtration rate. HbA1C, glycosylated haemoglobin. SBP, systolic blood pressure. DBP, diastolic blood pressure.

Table 4. Multivariate models* explaining prevalent cardiovascular disease in the ET2DS

	OR 95%CI	P value
Ferritin (Z score of log-ferritin)	**0.81(0.68-0.96)**	**0.018**
Ferritin (µg/L)		
Quintile 1	1.0(Reference)	
Quintile 2	0.80(0.48-1.32)	0.394
Quintile 3	0.50(0.30-0.83)	0.008
Quintile 4	0.65(0.39-1.09)	0.105
Quintile 5	0.62(0.37-1.04)	0.073
Sex (male as reference)	**0.50(0.35-0.71)**	**<0.001**
Fibrinogen µg/L	**1.32(1.04-1.68)**	**0.018**
DBP mmHg	**0.96(0.94-0.98)**	**<0.001**
HDL-cholesterol (mmol/L)	**0.30(0.18-0.52)**	**<0.001**
Anti-hypertensive treatment	**5.13(2.55-10.30)**	**<0.001**
Glitazone treatment	**0.50(0.31-0.78)**	**0.003**
Aspirin treatment	**3.51(2.38-5.18)**	**<0.001**

* Models on the basis of variables selected from a previous univariate analysis (P<0.1) and which remained significant in the multivariate model after a backward elimination approach (P<0.05).

The variables tested in the univariate analysis were the following: age (at baseline), sex, duration of diabetes, use of specific anti-hyperglycaemic agents, treatment with insulin, lipid-lowering drugs, blood pressure-lowering drugs, smoking(ever/never), alcohol consumption (ever/never), body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), HbA1c, fasting glucose, HDL-cholesterol, total cholesterol, C reactive protein (CRP), fibrinogen, liver enzymes, liver disease, estimated glomerular filtration rate (eGFR), haemoglobin levels, and aspirin treatment. Anaemia was alternatively tested instead of haemoglobin levels.

** Value not added because the correspondent variable was not significant in the univariate analysis (P<0.1) or did not remain significant in the multivariate analysis (P<0.05).

CI, confidence interval. CRP, C reactive protein. GGT, gamma-glutamyl transpeptidase. ALT, Alanine aminotransferase. eGFR, estimate glomerular filtration rate. DBP, diastolic blood pressure.

Table 5. Multivariate models* explaining incident cardiovascular disease in the ET2DS

	Incident cardiovascular disease (n=821)		Incident cardiovascular disease (n=536) –Excluding CVD cases at baseline	
	HR 95%CI	**P value**	**HR 95%CI**	**P value**
Ferritin (Z score of log-ferritin)	0.86(0.72-1.04)	0.136	0.77(0.60-1.01)	0.062
Ferritin (µg/L)				
Quintile 1	1.0(Reference)		1.0(Reference)	
Quintile 2	**0.50(0.28-0.90)**	**0.022**	**0.37(0.15-0.91)**	**0.031**
Quintile 3	**0.58(0.34-0.98)**	**0.046**	0.74(0.37-1.50)	0.415
Quintile 4	0.76(0.45-1.28)	0.305	0.56(0.26-1.21)	0.145
Quintile 5	**0.46(0.26-0.83)**	**0.010**	**0.34(0.14-0.81)**	**0.016**
Age (years)	**1.05(1.01-1.10)**	**0.010**	**1.08(1.01-1.15)**	**0.015**
Duration of diabetes (years)	**	**	**4.38(1.77-10.8)**	**0.001**
CRP mg/L	**	**	**1.82(1.05-3.14)**	**0.031**
GGT(U/L)	**2.64(1.57-4.43)**	**<0.001**	**2.83(1.44-5.56)**	**0.002**
ALT(U/L)	**0.21(0.06-0.77)**	**0.019**	**	**
HDL-cholesterol (mmol/L)	**0.50(0.28-0.91)**	**0.024**	**	**
eGFR, mL/min/1.73 m^2	**0.98(0.96-0.99)**	**0.007**	**	**
Insulin therapy	**1.87(1.22-2.87)**	**0.004**	**	**
CVD at baseline	**1.55(1.07-2.24)**	**0.019**	***	***

* Models on the basis of variables selected from a previous univariate analysis (P<0.1) and which remained significant in the multivariate model after a backward elimination approach (P<0.05).
The variables tested in the univariate analysis were the following: age (at baseline), sex, duration of diabetes, use of specific anti-hyperglycaemic agents, treatment with insulin, lipid-lowering drugs, blood pressure-lowering drugs, smoking(ever/never), alcohol consumption (ever/never), body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), HbA1c, fasting glucose, HDL-cholesterol, total cholesterol, C reactive protein (CRP), fibrinogen, liver enzymes, liver disease, estimated glomerular filtration rate (eGFR), haemoglobin levels, and aspirin treatment. Anaemia was alternatively tested instead of haemoglobin levels. CVD at baseline was covariate for the total cohort (n=821).
** Value not added because the variable was not significant in the univariate analysis (P<0.1) or did not remain significant in the multivariate analysis (P<0.05).
*** This variable does not apply for this model.

CI, confidence interval. CRP, C reactive protein. GGT, gamma-glutamyl transpeptidase. ALT, Alanine aminotransferase. eGFR, estimate glomerular filtration rate. DBP, diastolic blood pressure. CVD, cardiovascular disease. The values of CRP, transaminases levels, glucose and total cholesterol were log-transformed for this analysis.